addition, Applicants submit herewith in paper copy and on floppy disk the Sequence Listing in computer readable form. Applicants respectfully request entry of this Second Preliminary Amendment prior to examination of the present application.

Dated: February 14, 2002

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APPENDIX I
MARKED-UP VERSION OF SPECIFICATION'S REPLACEMENT PARAGRAPHS

The following is a marked-up version of the specification's replacement paragraphs pursuant to 37 C.F.R. §1.121(b) with markings showing changes made herein to the previous version of record of the specification.

IN THE SPECIFICATION

On page 37, please delete the paragraph beginning on line 13 and ending on line 20, and replace with the following paragraph:

A variety of mammalian cell lines may be employed for the expression of recombinant proteins according to the methods of the [resent]present invention. Exemplary cell lines include CHO cell lines [*e.g.*, CHO-K1 cells (ATCC CCl 61; ATCC CRL [9618)and]9618) and derivations thereof such as DHFR$^-$ CHO-KI cell lines (*e.g.*, CHO/dhFr-; ATCC CRL 9096), mouse L cells and BW5147 cells and variants thereof such as BW5147.3 (ATCC TIB 47) and BW5147.G.1.4 cells (ATCC TIB 48). The cell line employed may grow attached to a tissue culture vessel (*i.e*, attachment-dependent) or may grow in suspension (*i.e.*, attachment-independent).

On page 40, please delete the paragraph beginning on line 28 and ending on page 41, line 12, and replace with the following paragraph:

To generate a stable cell line expressing large quantities of a desired protein(s), the following vectors are introduced as linear DNA: 1) a selectable vector such as pMSD5-HPRT; 2) an amplifiable vector such as pSSD7-DHFR and 3) one or more vectors encoding a gene of interest. This also results in a much higher [ration]ratio of copies of the expressed gene(s) of interest to amplifiable marker genes in the concatemer. The ratio of the selectable vector, amplifiable vector and the vector(s) encoding a protein(s) of interest is 1:2:20-50. Multiple vectors encoding separate proteins of interest are utilized when it is desirable to express multiple proteins in a single cell. This will be the case where the protein of interest is a multi-chain protein. For example, immunoglobulins are formed by the association of two heavy chains and two light chains; the heavy and light chains are encoded by separate genes.

Expression of a functional immunoglobulin requires that the transfected cell express both the heavy and light chain genes. Up to six non-selectable/amplifiable plasmids (*i.e.*, encoding a gene of interest) may be used to transfect a given cell line.

On page 85, please delete the paragraph beginning on line 22 and ending on page 86, line 26, and replace with the following paragraph:

The 5' primer used to amplify TCR sequences contains the following restriction sites at the 5' end of the primer: *Xba*I, *Eco*RI and *Mlu*I followed 18-21 nucleotides comprising a consensus sequence derived from the V regions of human TCRs. Therefore the 5' primer will comprise sets of degenerate primers having the following sequence: 5'-TCTAGAATTCACGCGT$(N)_{18\text{-}21}$-3' (SEQ ID NO:80), where N is any nucleotide and the 18-21 nucleotide stretch represents a consensus V region sequence. The following 3' primer is used in conjunction with the above-described consensus 5' primer to amplify the extracellular domains of human TCR α chains: 5'-CGATCGTGGATCCAAGTTTAGGTTCGTATCTGTTTCAAA-3' (SEQ ID NO:35). The 3' connection for the TCR α chain is made after the asparagine which appears at position 110 of the constant (C) region of the α chain. The following 3' primer is used in conjunction with the above-described consensus 5' primer to amplify the extracellular domains of human TCR β chains: 5'-CGATCGAGGATCC AAGATGGTGGCAGACAGGACC-3' (SEQ ID NO:36). The 3' connection for the TCR α chain is made after the isoleucine which appears at position 147 of the C region of the β chain. These 3' primers are designed such that in both cases (*i.e.*, for both the α and the β chain of the TCR) the connection between the extracellular domains of the TCR with the thrombin site is made at the fourth amino acid residue from the apparent beginning of the respective transmembrane regions of the TCR chains. Both 3' primers contain recognition sites for *Pvu*I and *Bam*HI at their 5' ends. The restriction sites located at the 5' ends of the primers allows the resulting PCR products comprising a TCR chain to be removed as a *Xba*I or *Eco*RI or *Mlu*I (5' end)-*Bam*HI or *Pvu*I (3' end) fragment and joined with the appropriate thrombin-transmembrane DNA sequence [as a *Bam*HI or *Pvu*I (5' end)-*Not*I (3' end) fragment] and inserted into any of the SD7 vectors (*e.g.*, pSRαSD7). The resulting expression vectors (one for each of the α chains and the β chains of the chimeric TCR) are co-transfected using electroporation into BW5147.G.1.4 cells

along with the amplification vector pSSD7-DHFR (Ex. 3) and the selection vector pMSD5-HPRT (Ex. 2). The amount of each plasmid DNA to be used (the plasmids are linearized before electroporation), the conditions for electroporation, selection and amplification are described above. The resulting amplified cell lines will express the chimeric TCR heterodimer on the surface of the cell. The TCR is solubilized by digestion of the cells with thrombin. The thrombin solubilized extracellular domains will have 3 (TCR β) or 4 (TCR α) novel amino acids at the C-termini.

On page 90, please delete the paragraph beginning on line 8 and ending on line 16, and replace with the following paragraph:

Two micrograms of pSRαSD7 (Ex. [X]1) is cut with *Sal*I and *Hin*dIII (NEB enzymes, buffers & conditions). The plasmid is spermine precipitated (Ex. 5) and resuspended in 34 µl H_2O and 4 µl of 10x T4 DNA ligase buffer. Equal molar amounts (6.3 ng each) of the unphosphorylated oligonucleotides SXAPH5 (SEQ ID NO:42) and SXAPH3 (SEQ ID NO:43) are added. The reaction is chilled on ice, 400 units of T4 DNA ligase is added and the tube is placed at 14°C overnight. The ligation is transformed into bacteria and clones screened for the presence of the added *Asc*I & *Pac*I restriction sites. The resulting plasmid is called pSRαSD9. Figure 21 provides a schematic map of pSRαSD9.

On page 91, please delete the paragraph beginning on line 25 and ending on page 92, line 8, and replace with the following paragraph:

pM-HPRT-SSD9-DHFR contains the *hprt* gene under the control of the Moloney enhancer/promoter and the *dhfr* gene under the control of the SV40 enhancer/promoter. pM-HPRT-SSD9-DHFR is constructed by first subcloning the HPRT cDNA (Ex. 2) into pMSD8 (described below) to create pMSD8-HPRT. The small DNA fragment located between the *Sal*I and *Hin*dIII sites on pMSD8-HPRT is then replaced with a sequence containing *Asc*I and *Pac*I sites as follows. pMSD8-HPRT is digested with *Sal*I and *Hin*dIII and the SXAPH5 and SAXPH3 oligonucleotides (SEQ ID NOS:42 and 43) are ligated to the ends of the digested pMSD8-HPRT (as described in section i above) to create pMSD9-HPRT. The ~2450 bp *Sal*I-*Cla*I fragment containing the *Asc*I and *Pac*I sites, the Moloney enhancer/promoter, the HPRT cDNA and the EF1α poly A region is inserted between the *Sal*I and *Cla*I sites of pSSD7-

DHFR (Ex. 3) to generate pM-HPRT-SSD9-DHFR. Figure 26 provides a map of pM-HPRT-SSD9-DHFR.

On page 92, please delete the paragraph beginning on line 9 and ending on line 24, and replace with the following paragraph:

pMSD8 is similar to pMSD5 but contains the poly A site from the human elongation factor 1α gene. pMSD8 was constructed as follows: A 292 bp fragment containing the poly A site from the human elongation factor 1α gene [SEQ ID NO:78] was isolated from MOU cell (GM 08605, NIGMS Human Genetic Mutant Cell Repository, Camden, NJ) genomic DNA using PCR. MOU genomic DNA was isolated using conventional techniques. The PCR was conducted using 10 μg MOU genomic DNA and 1 μM final concentration of each primer in a 400 μl reaction. Reaction conditions were 94°C for 1 minute, 60°C for 1 minute, 72°C for 1.5 minutes, 30 cycles. *Taq* DNA polymerase was obtained from Perkin-Elmer. The following oligonucleotides were used to prime the PCR: 5EF1αPolyA: 5' GAATTCTTTTTGCGTGTGGCAG 3' (SEQ ID NO:[79]78) and 3EF1αPolyA: 5' ATCGATATTCCTTCCCCTTCC 3' (SEQ ID NO:[80]79). The 3EF1αPolyA oligonucleotide generates a *Cla*I site at the 3' end of the poly A site and the 5EF1αPolyA oligonucleotide generates an *Eco*RI site at the 5' end of the poly A site. Digestion of the PCR product with *Eco*RI and *Cla*I yields a 292 bp *Eco*RI/*Cla*I fragment.

Please replace the Sequence Listing filed 08/09/01 as pages 106-142 with the Substitute Sequence Listing submitted herewith as pages --106-143--.

IN THE CLAIMS:

Please renumber the Claims pages from pages "143-146" to --144-147--.

IN THE ABSTRACT OF THE DISCLOSURE:

Please renumber the Abstract page from page "147" to --148--.